Chattagram Maa-O-Shishu Hospital Medical College Journal
Volume 16, Issue 1, January 2017

Original Article

Detection and Antibiotic Sensitivity Pattern of *Gardnerella vaginalis* Isolated from Bacterial Vaginosis Patients Attending Chittagong Medical College Hospital

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Abstract

Background: Now a day's bacterial vaginosis is an extremely common health problem for women in the world which causes many complications both in the pregnancy and non-pregnancy states. *G. vaginalis* is most important cause of bacterial vaginosis. **Methods:** A prospective observational study was conducted to detect *G. vaginalis* in bacterial vaginosis and their sensitivity patterns on patients attending at the outpatient Department of Gynaecology and Obstetrics of Chittagong Medical College Hospital, Chittagong. A total of 170 sexually active female in the age group of 15-45 years, with abnormal vaginal discharge were selected for the study. A detailed history and a thorough clinical examination of all the cases were done. **Results:**In this study 38(22.35%) *Gardnerella vaginalis* were isolated by culture and bacterial vaginosis was detected by different methods 47(27.65%). Antimicrobial resistance is one of the major public health threats. So antimicrobial sensitivity pattern of the organisms should be done at regular intervals. **Conclusion:** In this study *Gardnerella vaginalis* showed high (52.63%) resistant to most commonly used metronidazole and 100% sensitive to clindamycin.

Key words: Bacterial vaginosis; *Gardnerella vaginalis*; Amsel criteria; Nugent criteria; Human Blood Bilayer Tween 80 (HBT) agar media; Antibiotic sensitivity.

INTRODUCTION

Bacterial Vaginosis (BV) is a clinical syndrome characterized by shift of protective resident microorganisms as *Lactobacillus* spp. by opportunistic pathogenic bacteria such as *Gardnerella vaginalis* and other anaerobic bacteria. In most cases of BV, the predominant bacterial species found is *Gardnerella vaginalis*. Historically, *G. vaginalis* was thought to be the sole causative agent of this condition. But its role in the aetiology of BV was downgraded over the years. The biofilm-forming potential and cytotoxic activity of *G. vaginalis* have renewed interest in the virulence of this organism[1]. So bacterial vaginosis is mostly caused by the synergistic interaction of *G. vaginalis* with obligate anerobes.

Bacterial vaginosis is associated with many gynaecologic complications, such as cervicitis, salpingitis, endometritis, post-operative infections and pelvic inflmmatory disease, and many obstetric complications, such as premature rupture of the membranes, preterm deliveries, chorioamniotitis and postpartum endometritis. Bacterial vaginosis is also associated with an increased risk of HIV-1 transmission in non-pregnant women and more susceptible to *Herpes simplex virus, Chlamydia trachomatis, Neisseria gonorrhoe, and Human Papilloma Virus* (HPV) and post surgical infection[2].

Antibacterial resistance has become a major clinical concern worldwide including Bangladesh[3]. Extensive and indiscriminate use of antibiotics has created a major problem-drug resistance. The widespread and inappropriate use of antibiotics has resulted in the development of a progressively antibiotic-resistant microbial ecosystem in Bangladesh[4].

This study was designed to isolate the causative agent *G. vaginalis* from bacterial vaginosis patients with their antibiotic sensitivity pattern which would guide clinicians and microbiologists for proper handling of this pathogen & prevent unnecessary use of antibiotics.

MATERIALS AND METHODS

This was a prospective observational comparative study carried out in the Department of Microbiology, Chittagong Medical College, Chittagong, during the period of July' 2011 to June 2012. Approval from ethical review committee of Chittagong Medical College was duly taken. A total of 170 women, 50 pregnant and 120 non- pregnant, in the age group of 15-45 years patients attending the Gynae out-patient department of Chittagong Medical College Hospital was enrolled for this study. The results of the experiments were recorded systematically and statistical analysis was done by Statistical Package for Social Sciences (SPSS).

Inclusion criteria:

Women of reproductive age within 15-45 years, both pregnant and non pregnant, with abnormal vaginal discharge, with or without mild vulver itching or burning are considered as patients.

Exclusion criteria:

1. Below 15 yr & over 45yrs.
2. Known case of malignancy or AIDS patient.
3. History of taking antimicrobial agents or vaginal medication for vaginitis within the last one month.
4. Menstruating women.
5. Patient having history of vaginal douche on the day of examination.

Procedure:

Samples were collected with all aseptic precaution after taking informed consent from patient or her legal attendant. Three vaginal swab samples were collected from each patient by standard technique. First swab sample was collected from right vaginal wall and used for making Gram's stain, amine test and wet mount preparation. Second swab sample was collected from left lateral vaginal wall for culture of *Gardnerella vaginalis*. Third swab sample collected from vaginal fornix and used for new rapid BV assay test.

Detection of bacterial vaginosis by different clinical and microbiological methods :-

1. Amsel criteria
2. Nugent criteria
3. Bacterial vaginosis assay test
4. Isolation of *Gardenella vaginalis* by culture

Culture for isolation of Gardnerella vaginals:

The second swab inoculated into a selective and differential Human Blood Bilayer Tween 80 (HBT) agar media, Human blood agar media, Human blood Colombia agar media for isolation and subculture of *G. vaginalis*.

Procedure of culture:

Collected vaginal swab was inoculated and the plate was placed immediately in the candle extinction jar containing water soaked cotton. All plates are incubated in 5% Co_2 with increased humidity at 37^0 C for 48 - 72 hrs for primary isolation of *G. vaginalis* and read at 48 hours and rechecked at 72 hours before discarded. The plates were examined by oblique lighting after 24 hrs, 48 hrs, and 72 hrs.

Colonies on HBT agar media were identified as round opaque, smooth colonies that were pinpoint in size after 24 hrs of incubation and 0.5 mm in diameter at 48 hrs, produce β hemolysis after 48 or 72 hrs of incubation.

The β-hemolytic colonies from HBT agar were examined by Gram's staning to see Gram negative coccobacilli. Subcultures were done on Human blood Columbia agar media and Sheep blood agar media by using β-haemolytic colony for pure isolation and to see the haemolytic character. Colonies were also used for catalase test, oxidase test and fermentation of different sugar.

The identification of Gardnerella vaginalis, based on:-

1. Colonial morphology: Colonies on HBT agar were identified as small white colonies with β-hemolysis after 48 to 72 hours of incubation.
2. Clear β-hemolysis with diffuse edges on HBT media, but no hemolysis on sheep blood agar. The zone of hemolysis was 1to 2 mm wide around the isolated colonies on HBT agar after 48 hours of incubation.
3. Gram stained smear from a colony: Gram variable or Gram negative coccobacilli or small rods.
4. Catalase and oxidase test negative.
5. Fermentation of different sugur: - Maltose, mannitol, lactose, sucrose.
6. Susceptibility to different antimicrobial agents.

Antimicrobial susceptibility:-

All the isolates of *G. vaginalis* obtained by culture were tested for antimicrobial susceptibility by the single disc diffusion method against different antimicrobial agents. The organisms were tested against Metronidazole (MTZ) Clindamycine (CD) Ampicilin (AMP) Ceftriaxone (CRO) Erythromycin (E) Ciprofloxacin (CIP) Vancomycin (VA) Cotrimoxazole (SXT) Chloramphenicol (C) Tetracycline (TE).

Reference strain for quality control:-

The discs from each batch were standardized by testing against reference strains of *E. coli* ATCC 25922, *Staph. aureus* 25923 and zones of inhibition were tested with standard value.

RESULTS

A total of 170 women, 50 pregnant and 120 non- pregnant, clinically suspected cases of Bacterial Vaginosis (BV) aged between 15-45 years with abnormal vaginal discharge, with or without mild vulver itching or burning were included in this study.

Figure 1 : Out of 170 cases, on the basis of Amsel criteria (Clinical criteria) 43(25.30) cases were Bacterial Vaginosis (BV) positive and 127(74.70%) BV negative. On the basis of Nugent criteria 45(26.47%) were BV positive and 125(73.53%) BV negative. The results of BV assay test shows 46 (27.06%) cases were BV assay test positive and rest 124(72.94%) were negative.

Figure 2 : shows that culture of vaginal fluid yielded growth of *G. vaginalis* in 38(22.35%) cases and 132(77.65%) cases were culture negative.

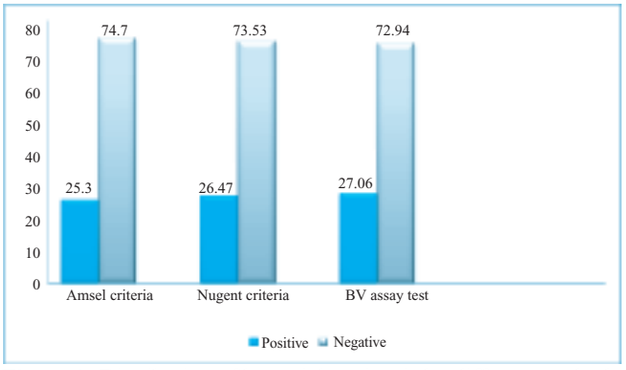

Figure 1: Distribution of bacterial vaginosis by different methods.

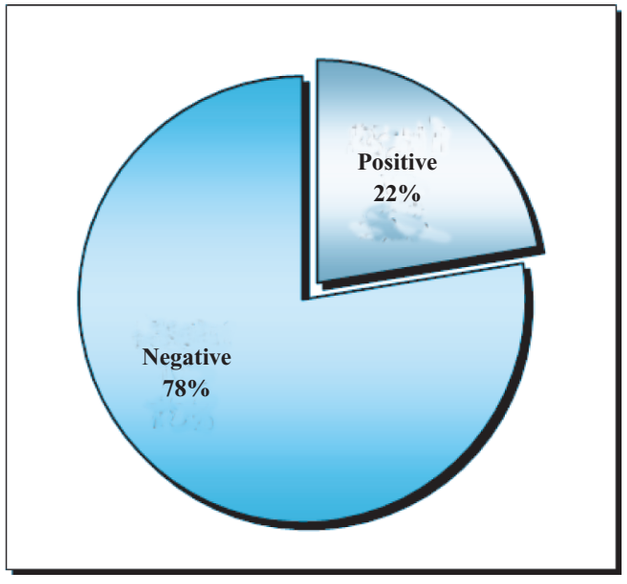

Figure 2 : Distribution of study population on the basis of culture of *G. vaginalis.*
(Pie chart –1: Distribution of culture of GV result)

Table I : The diagnosis of Bacterial Vaginosis (BV) by Amsel criteria, Nugent criteria, BV assay test and culture of *G. vaginalis* are shown in Table I. Out of all 47 BV cases, 38 cases positive by culture were also positive by other three methods. Five more BV cases were positive by these three methods. Additional 02 cases were positive by only BV assay test. One case was positive by both Nugent criteria and BV assay test. Another one case was positive by only Nugent criteria.

Table I : Detection of bacterial vaginosis cases by combined methods (n =170).

Different Methods	BV Positive	Percentage (%)
Amsel & Nugent Criteria, Rapid BV Assay and culture All four tests positive	38	22.35
Amsel , Nugent Criteria and Rapid BV Assay Three tests positive -culture negative	05	2.94
Nugent Criteria, Rapid BV Assay test positive & Amsel criteria, culture negative	01	0.59
Only rapid BV Assay test positive & other three test negative	02	1.18
Only Nugent Criteria positive & other three test negative	01	0.59
Total (170)	**47**	**27.65**

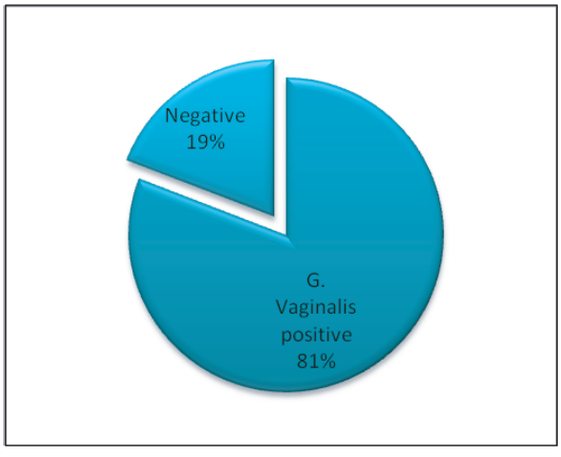

Figure 3 : Distribution of isolated *G. vaginalis* among the total bacterial vaginosis (n=47)
(Pie chart: *G. vaginalis* in Bacterial vaginosis)

Table 2 Sensitivity pattern of 38 isolates shows that G. vaginalis were 81.58% resistant to tetracycline, 78.95% to cotrimoxazole, 68.42% to ciprofloxacin, 52.63% to metronidazole and 26.32% to erythromycin, while 100% sensitive to clindamycine followed by vancomycin (94.74%) ceftriaxone (86.84%) ampicillin (78.95%) chloramphenicol (73.68%).

Table 2 : Antimicrobial susceptibility pattern of *Gardnerella vaginalis* isolates (n = 38).

Antimicrobial agent	Sensitive	Intermediate sensitive	Resistance
Clindamycin (CD)	38 (100.00)	00 (0.00)	00 (0.00)
Vancomycin (VA)	36 (94.74)	02 (5.26)	00 (0.00)
Ceftriaxone (CRO)	33 (86.84)	04 (10.53)	01 (2.63)
Ampicillin (AMP)	30 (78.95)	06 (15.79)	02 (5.26)
Chloramphenicol (C)	28 (73.68)	06 (15.79)	04 (10.53)
Erythromycin (E)	23 (60.52)	05 (13.16)	10 (26.32)
Metronidazole (MTZ)	14 (34.21)	04 (10.53)	20 (52.63)
Ciprofloxacin (CIP)	08 (21.05)	04 (10.53)	26 (68.42)
Cotrimoxazole (SXT)	04 (10.53)	04 (10.53)	30 (78.95)
Tetracycline (TE)	04 (10.53)	03 (07.89)	31 (81.58)

Figures within parentheses indicate percentages.

DISCUSSION

Bacterial vaginosis is the most common infection in female worldwide leading to vaginal disorders. It may be a polymicrobial syndrome but recent studies have shown *Gardnerella vaginalis (G. vaginalis)* also can be a primary pathogen in half of the cases of bacterial vaginosis. This study was primarily designed to detect the *G. vaginalis* among the BV patients with their sensitivity pattern to decrease the drug resistant.

In the present study, the detection rate of BV by Amsel criteria, Gram stain Nugent criteria and BV assay test was 43(25.30%) 45(26.47%) and 46(27.06%) respectively. It correlates with findings of Begum et al, Akhter et al where BV was 24% and 21.5% by Amsel criteria, 23% and 21% by Gram stain Nugent criteria respectively. On the oher hand, Millar and Posner et al[5-8]. found bacterial vaginosis by BV assay test were 39% and 30% respectively.

In this study vaginal specimen from study cases were subjected to culture in Human Blood Bilayer Tween (HBT) agar media , a highly selective media, yielded growth of *G. vaginalis* from 22.35% of total study cases. The isolation was higher than that of Devi et al and Udayalaxmi in India who reported 17.42% and 16.7% respectively, but lower than that of Gupta et al in India and and Totten et al in Belgium who reported 54.1% and 91% respectively[9-12]. Begum et al, Akhter et al from BSSMU in Bangladesh reported similar findings 25.5% and 21% respectively[5-6].

This slightly higher rate reported by Gupta et al and Totten et al might be due to the use of three or more media that were either non selective or enriched for primary isolation of *G. vaginalis* and variable methods for their identification[11-12].

In this study total Bacterial Vaginosis (BV) was 47(27.65%) by Amsel criteria, Nugent criteria, BV assay test and culture of *G. vaginalis*. Out of all 47 BV cases, 38 cases positive by culture were also positive by other three methods. Five more BV cases were positive by these three methods. Additional 02 cases were positive by only BV assay test. One case was positive by both Nugent criteria and BV assay test. Another one case was positive by only Nugent criteria. This findings correlates with the Bilkis et al (22.65%) Akhter et al (23%) in Bangladesh and Puri et al (31%) in India. But Bhalla et al And Mohadani et al found high prevalence 50% and 51% respectively[13,6,14,15,16]. Factors responsible for higher prevalence of bacterial vaginosis among the study population were lower socio-economic status, improper sanitation, poor hygiene, malnutrition and might be attributed to non-inclusion of clue cells in their study[6]. Slightly lower incidence in our study may be due to mandatory inclusion of clue cells on saline wet mount and gram stain criteria as a marker of BV for every case, which makes the results more specific.

Gardnerella vaginalis is present in up to 95% of cases of BV[17]. But in this study we isolated G. vaginalis 81% from BV patient. Gardner & Dukes isolate *G. vaginalis* 92% of woman with BV and Amsel isolated *G. vaginalis* 96% from BV patient[18,19]. In our study we used highly selective media, which might be influence the higher growth.

The antibiotic sensitivity pattern of *G. vaginalis* showed extreme variation in different studies. This variation could be attributed to variation in disc potency, media used for susceptibility testing and incubation environment (Aerobic or anaerobic). But there is no difference in opinion regarding use of metronidazole and clindamycin. Both this drugs were found most sensitive in various previous studies[13]. But in our study metronidazole was 52.63% resistant. Similarly Nagaraja et al and Goldstein et al reported higher resistant to metronidazole[20,21]. Nagaraja reported 68% resistant to metronidazole. Antibiotic resistance is being increasing day by day around the world.

The sensitivity results show that clindamycin (100%) vancomycin (94.74%) and ceftriaxon (86.84%) were highly sensitive but vancomycin and ceftriaxon were not used for treatment of BV. The next effective drugs were ampicillin (78.95%) chloramphenicol (73.68%) and erythromycin (60.52%). In spite of the higher sensitivity of chloramphenicol and erythromycin, these drugs are not commonly used, because of the toxicity in case of chloramphenicol and inefficiency of erythromycin in acid pH in vagina[22]. Ampicillin was 78.95% sensitive against *G. vaginalis* in this study, but was not so effective in vivo. The use of ampicillin for the treatment of

bacterial vaginosis has often been associated with failure to eradicate *G. vaginalis* or clinical cure. This is probably due to inactivation of ampicillin by the β-lactamases produced by vaginal anaerobes. However, this agent may have a role in treating *Gardnerella*-associated infections at extravaginal sites[22]. Ampicillin also inhibits the growth of *Lactobacilli,* so prevents recolonization by this organism after therapy[23].

In our study other effective drug was ciprofloxacin, which was 21.05% sensitive. As ciprofloxacin is commonly used in UTI, it is expected that women associated with BV could have beneficial effect out of it. Other studies also do not encourage its use in BV.

The maximum resistance found in case of cotrimoxazole (78.95%) and tetracycline (81.58%) in this study, do not recommended its used in BV, though in the past they were used with controversial results[11].

Finally, several studies have evaluated the clinical and microbiological efficacy of metronidazole and clindamycin to treat BV and to prevent the recurrence of BV [21, 22]. In our study we found 100% sensitive to clindamycine but 68% resistant to metronidazole. So, to know the sensitivity pattern of the organisms at regular interval is important, particularly in developing countries where there is excessive use of antibiotics and lack of adequate antimicrobial resistance surveillance[3].

CONCLUSION

Though bacterial vaginosis is a global problem, we should detect the principle pathogen and treat them properly. Extensive and indiscriminate use of antibiotics has created a major problem- drug resistance. Several antimicrobial agents have been used to treat symptomatic BV. Until recently, the mainstay therapy consisted of either metronidazole or clindamycin. But in our study *Gardnerella vaginalis* showed high resistance to commonly used antibiotics metronidazole. So indiscriminate use of antibiotics should be avoided.

DISCLOSURE

All the authors declared no competing interest.

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